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METHOD DEVELOPMENT FOR THE ASSESSMENT OF POSSIBLE HUMAN EXPOSURE TO PESTICIDES AND INDUSTRIAL CHEMICALS

by

Thomas R. Edgerton
Robert F. Moseman
Lynn H. Wright

Analytical Chemistry Branch
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

U. S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

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16. ABSTRACT

The determination of chlorinated phenols in urine can be used as a means for assessing exposure to pesticides and industrial chemicals in the human population.

A method was developed for the analysis of chlorinated phenols which involves the derivatization of metabolites from the urine of rats fed hexachlorobenzene (HCB) and pentachlorophenol (PCP). This method was then applied to urine samples taken from the general human population to gain a background level. Pentachlorophenol was detected in greater than 90% of the human samples analyzed. The only other metabolites detected were tetrachloropyrocatechol and tetrachlorohydroquinone along with large amounts (greater than 3 ppm) of PCP. Pentachlorothiophenol, a major metabolite of HCB fed to rats, was not detected in human urine.

The analysis of human urine for underivatized chlorinated phenols using a direct gas chromatographic method not requiring derivatization detected quantifiable levels of 2,5-dichloro-, 2,4,5-trichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol in greater than 90% of the samples examined. Approximately 50% of the samples contained detectable levels of 2,6 and 3,5-dichlorophenol and 2,4,6-trichlorophenol.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report represents a research effort in the development of methods which will allow for assessment of humans to possible exposure to pesticides and industrial chemicals.

The emphasis of this project was to develop methodology for determining the metabolites of the chemicals from rat feeding studies and apply these developed methods to humans. Such data is required for the continued development of criteria for assessing human exposure to this group of chemicals.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The determination of chlorinated phenols in urine can be used as a means for assessing exposure to pesticides and industrial chemicals in the human population.

A method was developed for the analysis of chlorinated phenols which involves the derivatization of metabolites from the urine of rats fed hexachlorobenzene (HCB) and pentachlorophenol (PCP). This method was then applied to urine samples taken from the general human population to gain a background level. Pentachlorophenol was detected in greater than 90% of the human samples analyzed. The only other metabolites detected were tetrachloropyrocatechol and tetrachlorohydroquinone. The urine of a worker occupationally exposed to PCP exhibited quantifiable amounts of tetrachloropyrocatechol and tetrachlorohydroquinone along with large amounts (greater than 3 ppm) of PCP. Pentachlorothiophenol, a major metabolite of HCB fed to rats, was not detected in human urine.

The analysis of human urine for underivatized chlorinated phenols using a direct gas chromatographic method not requiring derivatization detected quantifiable levels of 2,5-dichloro-, 2,4,5-trichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol in greater than 90% of the samples examined. Approximately 50% of the samples contained detectable levels of 2,6 and 3,5-dichlorophenol and 2,4,6-trichlorophenol.

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SECTION 1

INTRODUCTION

Chlorinated phenols have been of concern to the enivronmental scientist for many years. Their toxicity to fish and other aquatic life is well documented.[1,2] The presence of chlorophenols in industrial effluents has also been demonstrated.[3] Methodology was developed to analyze for chlorinated phenols in urine to assess possible human exposure to chemicals known or suspected to give these compounds as metabolites.[4,5,6]

The most widely used and studied chlorinated phenol is pentachlorophenol (PCP). It has been used extensively in the wood products industry as a preservative and in agriculture as a fungicide. A discussion of its uses, toxicity, and fate in the environment is found in the literature.[7] The occurrence of PCP in humans from occupational exposed workers and the general human population is also well documented.[8,9]

The metabolism of PCP and other chemicals which give rise to PCP and other chlorinated phenols as metabolites can be found in the literature.[10-16] A listing of possible sources of chlorinated phenols is found in Table 1. This list is by no means complete, but does give some insight into possible origins of various chlorinated phenols that might be encountered in exposure assessment work.

Table 1. Possible Origins of Various Chlorinated Phenols

Metabolite	Origin	Type Pesticide
2,6-dichlorophenol	Lindane	Insecticide
2,4-dichlorophenol	Lindane	Insecticide
	VC-13	Insecticide
	m-dichlorobenzene	Fumigant
	2,4-D	Herbicide
2,3-dichlorophenol	Lindane	Insecticide
	o-dichlorobenzene	Fumigant
2,5-dichlorophenol	Lindane	Insecticide
	2,4,5-T	Herbicide
	p-dichlorobenzene	
3,4-dichlorophenol	Lindane	Insecticide
	o-dichlorobenzene	Fumigant
	Diuron	Herbicide
3,5-dichlorophenol	Lindane	Insecticide
	PCP	
2,3,4-trichlorophenol	Lindane	Insecticide
2,3,5-trichlorophenol	Lindane	Insecticide
	PCP	
2,3,6-trichlorophenol	Lindane	Insecticide
2,4,5-trichlorophenol	Lindane	Insecticide
	Ronnel	Insecticide
	Erbon	Herbicide
	2,4,5-T	Herbicide
	HCB	Fungicide
	Tetrachlorvinphos	
2,4,6-trichlorophenol	Lindane	Insecticide
3,4,5-trichlorophenol	Lindane	Insecticide
2,3,5,6-tetrachlorophenol	HCB	Fungicide
2,3,4,6-tetrachlorophenol	PCP (impurity)	Fungicide
	Lindane	Insecticide
2,3,4,5-tetrachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
pentachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
	PCNB	Fungicide

Metabolism studies and analytical methods development were conducted using rats fed two chemicals, pentachlorophenol (PCP) and hexachlorobenzene (HCB), which were known or suspected to metabolize to chlorinated phenols. The method was then applied to urine samples from the general human population and the urine of a worker occupationally exposed to pentachlorophenol.

The literature contains no apparent methodology for the determination of lower chlorinated phenols in urine at the low ng/g level. Because of this, a method was developed in an attempt to detect mono-, di-, and trichlorophenols in urine. No metabolism studies were performed, but the method was applied to general human population urine samples.

SECTION 2

CONCLUSIONS

Pentachlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol were found in greater than 90% of the human urine samples analyzed. Since all but one of the samples was from the general population, it is difficult to determine a common exposure route. The urine of workers occupationally exposed to other chemicals which can give chlorinated phenol metabolites and further animal feeding studies need to be conducted before definitive exposure assessments can be made.

The determination of chlorinated phenols in urine can possibly be used as an index of exposure to numerous chemicals.

SECTION 3

MATERIALS AND METHODS

HEXACHLOROBENZENE AND PENTACHLOROPHENOL[17]

Animals

Adult female Sherman rats, 3-4 months of age and weighing 215-275 g, were distributed into the following treatment groups: group 1, 4 rats on 575 ppm cornstarch in chow; group 2, 6 rats on 100 ppm HCB; group 3, 6 rats on 100 ppm PCP. The HCB and PCP diets were prepared by mixing a quantity of HCB or PCP with a small amount of cornstarch (resulting in a starch concentration of about 575 ppm in the final mix) in a mortar, then making the appropriate dilution with ground chow and mixing in an electric mixer.

Rats were housed 2 per cage, identified with ear tags, and provided their respective diets and water ad libitum. Animals were weighed weekly and food consumption measured at least 2 days each week. Individual urine samples were collected overnight, after 30 days and after 107 days. Rats were fed plain chow during urine collection to avoid non-ingested parent compound in the collected urine.

No clinical signs of toxicity were observed. Weight gain and food consumption were comparable in all rats. Average HCB or PCP ingestion over the first 30 days was 6.5 mg/Kg/day for both compounds.

Apparatus

Tracor MT-220, gas chromatograph equipped with a nickel-63 electron-capture detector, was operated in the pulsed linearized mode. Borosilicate glass columns (1.8 m x 4 mm i.d.) were packed with 80/100 mesh Gas Chrom Q coated with 5% OV-210, 3% OV-1, 3% Silar 10-C, 4% SE-30/6% OV-210 or

1.5% OV-17/1.95% QF-1. A 5% DEGS coated on 80/100 mesh Gas Chrom P was also used. The OV-1, Silar 10-C, SE-30/OV-210, and OV-17/QF-1 columns were operated at 170°C with a 5% methane in argon carrier gas flow rate of 60 mL/min. The OV-210 column was operated at 160°C with a 5% methane in argon flow rate of 40 mL/min. The DEGS column was operated at 170° with a 5% methane in argon flow rate of 90 mL/min. Detector, inlet, and transfer line temperatures were 300°, 235°, and 220°, respectively.

Analytical results were confirmed on a Finnigan Model 3200 quadrupole mass spectrometer equipped with a Model 9500 Gas Chromatograph and Model 6100 Data System. Methane was used as the reagent gas for operation in the chemical ionization mode with a source temperature of 120°C, pressure 117 Pa, 110 eV electron energy and 1.0 ma emission current.

For gas chromatography-mass spectrometry (GC/MS), a borosilicate glass column (1.2 m x 2 mm i.d.) was packed with 80/100 mesh Gas Chrom Q coated with 5% OV-210. The column was operated at 90°C isothermal for 1 minute, then programmed at 4°C per minute to a final temperature of 160°C. Methane carrier gas flow rate was 20 mL/min. Inlet, transfer line, and ion source temperatures were 200, 250, and 120°C, respectively.

Reagents and Materials

Anhydrous, granular sodium sulfate and sodium bisulfite were Soxhlet extracted for 4 h with hexane and oven dried at 130°.

Acid alumina, Brockman Activity I (Fisher Scientific), was dried for 24 h at 130°C and stored in a desiccator.

Potassium hydroxide and hydrochloric acid were reagent grade.

N-Methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.), should be handled carefully since it is a known carcinogen.

All solvents were pesticide quality or equivalent.

2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, pentachlorophenol, pentachlorothiophenol, 2,3,4,5-tetrachlorophenol, were obtained from Aldrich Chemical Co.; tetrachloropyrocatechol from Pfaltz and Bauer, Inc., Flushing, NY; and tetrachlorohydroquinone from K&K Laboratories, Inc., Plainview, NY. Pentachlorothiophenol, tetrachlorohydroquinone and tetrachloropyrocatechol were recrystallized prior to use.

The purity of HCB (based on EC-GC) used for dietary feeding was greater than 99.5%. No apparent impurities were found by EC-GC analysis. PCP contained 0.8% 2,3,4,6-tetrachlorophenol as the only detectable impurity.

Methylating Reagent

Potassium hydroxide (2.3 g) was dissolved in 2.3 mL of distilled water in a 125 mL Erlenmeyer flask and cooled to room temperature. Twenty-five milliliters of ethyl ether was then added and the flask was cooled in a refrigerator. The following step was carried out in a glove box or high-draft hood. N-Methyl-N'-nitro-N-nitrosoguanidine (1.5 g) was added in small portions to the flask with vigorous shaking. The ether layer, which contained diazomethane, was decanted into a scintillation vial and stored in a freezer.

Preparation of Standard Solutions

A standard of each phenol was prepared in hexane and stored at -15°C in brown glass bottles. Urine fortifications were made from

acetone dilutions of the seven mixed phenol standards. A volume containing 10 µg of each phenol was pipeted into separate 15 mL graduated centrifuge tubes. Methylation was accomplished by adding 5 mL of diazomethane reagent in a high draft hood to each tube (CAUTION: Diazomethane is toxic and may be explosive under certain conditions). The phenol standards were allowed to stand for 30 minutes before EC-GC determination. Nitrogen was bubbled through the individual standard solutions to remove any excess diazomethane prior to EC-GC analysis. For determination of elution patterns on an acid alumina column, a known amount of each phenol was methylated as a mixture, and allowed to stand for 30 minutes. The methylated phenol mixture was concentrated to 0.2 to 0.3 mL under a gentle stream of nitrogen prior to being placed on the acid alumina column.

Preparation and Elution of Acid Alumina Column

A size 22-9 chromaflex column (Kontes 420530) was loosely plugged with a small amount of glass wool. Acid alumina (4 g) was added in small increments with tapping. Anhydrous, granular Na_2SO_4 (1.6 g) was added on top of the alumina. Thirty milliliters of 40% benzene in hexane was used to wash the column free of interferences. After thoroughly air drying, the column was placed in an oven at 130°C overnight prior to use.

A prepared column was removed from the oven just prior to use and allowed to cool to room temperature. The column was wetted with 7 mL of hexane. When the solvent layer reached the top of the Na_2SO_4, an aliquot of methylated sample or methylated standard phenol mixture in 0.2 to 0.3

mL was placed on top of the column. Quantitative transfer of the sample or standard was accomplished with three 0.5 mL rinsings with hexane. An additional 3.5 mL of hexane was added and the hexane fraction (5.0 mL) was collected and discarded. 2,3,4,6-Tetrachlorophenol, 2,3,5,6-tetrachlorophenol, pentachlorophenol, and pentachlorothiophenol were eluted with 20 mL of 10% benzene in hexane (Fraction I). The remaining phenols, 2,3,4,5-tetrachlorophenol, tetrachloropyrocatechol, and tetrachlorohydroquinone, were eluted with 20 mL of 40% benzene in hexane (Fraction II). Fractions I and II were adjusted to an appropriate volume for injection into the gas chromatograph.

Analysis of Urine

Two milliliters of urine were transferred into a 20 mm x 125 mm Teflon® lined screw cap culture tube and 100 mg of sodium bisulfite was added. The urine was acidified by the addition of 0.5 mL of concentrated HCl. The tube was sealed and placed in a boiling water bath for 1 h with periodic shaking. After hydrolysis, an additional 100 mg of sodium bisulfite was added to the cooled urine sample and extracted twice for one hour each on a mechanical rotator at 30 to 50 RPM using two 5 ml portions of benzene. The samples were centrifuged after each extraction and the extracts combined in an aluminum foil wrapped 15 mL centrifuge tube. The benzene extract was concentrated to a volume of 0.3 to 0.5 mL in a water bath at 30°C under a gentle stream of nitrogen and methylated with 5 mL of diazomethane reagent. The methylated extract was allowed to stand for 1 h. Prior to column cleanup, the methylated urine extract was concentrated to approximately 0.3 mL under a gentle stream of nitrogen. Two milliliters of hexane were added, and the solution was again reconcentrated to a volume of 0.3 mL.

UNDERIVATIZED CHLORINATED PHENOLS

Apparatus

A Tracor MT-222 gas chromatograph, equipped with a nickel-63 electron capture detector, was operated in the pulsed linearized mode. Borosilicate glass columns (1.8 m x 4 mm I.D. or 0.6 m x 4 mm I.D.) were packed with double support-bonded diethylene glycol succinate (DSB-DEGS) or support-bonded butane 1,4-diol succinate (SB-BDS) on 80/100 mesh acid washed Chromosorb W.[18,19] The columns were operated at 180°-210° with a 5% methane in argon carrier gas flow-rate of 60-80 mL/min. Other temperatures were: detector, 300°; inlet 225°; transfer line, 220°.

The following glassware was used: Chromaflex column, plain, teflon stopcock, 250 x 10.5 mm I.D., (Kontes Cat. No. K-420280); Kuderna-Danish concentrator assembly (K-570000); 25-ml graduated tubes, size 2525 (K-570050); 15 x 125 mm screwcap culture tube.

Reagents and Materials

Chlorinated phenol reference standards were obtained from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A). For fortification purposes, the phenols were converted to their respective sodium salts prior to addition to urine.

All solvents were pesticide quality or equivalent.

Reagent materials (3 N HCl, 0.1 N NaOH, $NaHSO_3$, deionized water) were extracted with hexane and toluene prior to use.

The macroreticular resin, XAD-4, was obtained from Rohm and Haas (Philadelphia, Pa., U.S.A.). The fines were removed by slurrying in methanol and decanting.[20] The remaining beads were purified by Soxhlet extraction with 3 N HCl for 18 h followed by neutralization with

water in a Buchner funnel. The neutralized resin was washed with six 50 mL volumes of 0.1 N NaOH. The resin was again neutralized with water and allowed to dry. The dried resin was then sequentially extracted with methanol, acetonitrile, acetone and hexane in a Soxhlet extractor for 8 h per solvent.[20] The purified resin was stored under methanol in a glass stoppered bottle.

Preparation of XAD-4 Column

A small plug of hexane-extracted glass wool was placed in a 250 mm x 10.5 mm I.D. chromaflex column. Approximately 6 cm (1.5-2 gm dry weight) XAD-4 resin in methanol was added to the column. The flow of methanol was halted when the level of solvent reached the top of the resin bed. A second plug of glass wool was placed on top of the resin bed.

Preparation of Urine

Four milliliters of urine were placed in a 15 x 125 mm screw-cap culture tube. To this was added 100 mg of sodium bisulfite and 1 mL of concentrated HCl. The tube was sealed with a Teflon® lined screw-cap and placed in a boiling water bath for 1 h with periodic shaking. The sample was removed and cooled to room temperature.

Column Elution and Regeneration

The XAD-4 column was rinsed with 10 ml of deionized water. When the water level reached the top of the resin bed, 15 mL of 3 N HCl was added. After approximately 5 mL of acid eluted through the column, the flow was halted and the column allowed to equilibrate for 5 min. After equilibration the flow of acid was continued and a phenol standard or hydrolyzed urine sample was added to the column when the level of acid

reached the top of the resin bed. Quantitative transfer of the sample was accomplished with two 1 mL rinsings with 3 N HCl. The column was eluted with an additional 23 mL of 3 N HCl followed by 25 mL of deionized water. Both acid and water eluates were discarded. When the water level reached the top of the resin bed, a Kuderna-Danish (K-D) concentrator assembly was placed under the column and the phenols were eluted from the column with 100 mL of 10% 2-propanol in hexane (V/V). After the first 5 mL was eluted and collected, the flow was halted and the column allowed to equilibrate for 5 min. After equilibration, the flow was continued and the eluate collected. The K-D assembly was removed and the column was regenerated by washing with approximately 25 mL of methanol which was discarded. A volume of methanol was kept in the column until further use.

Concentration

The K-D assembly was placed on steam bath and the sample was concentrated to a final volume of 10-15 ml (two phases). The sample was cooled and the hexane layer (upper) transferred to a 15 ml centrifuge tube. The extract was then further concentrated to a volume of 1-2 ml for analysis by EC-GC.

CONFIRMATION TECHNIQUES

Results from the hexachlorobenzene and pentachlorophenol studies were confirmed by the GC/MS technique as was previously described.

Samples from the general chlorinated phenol method were confirmed by a separate gas chromatograph column (Butane diol succinate).

SECTION 4

RESULTS

HEXACHLOROBENZENE (HCB), PENTACHLOROPHENOL (PCP) STUDIES

Results of recovery studies for the suspected chlorinated phenol metabolites from HCB and PCP from fortified urine and through acid alumina column cleanup are given in Tables 2 and 3. Recovery of pentachlorothiophenol was low, possibly due to an oxidative reaction or to binding by components in urine. Difficulty was also encountered in analyzing for di- and trichlorophoenols using the derivatization technique because of the method's lack of reproducibility in derivatizing these compounds.

Retention times for the methyl ethers of the suspected chlorinated phenol metabolites found in HCB and PCP feeding study are listed in Table 4. A 5% OV-210 column was found to give adequate separation after column cleanup for these particular chlorinated [illegible] methyl ethers. A 5% DEGS column was used only for separation of 2,3,5,6 from 2,3,4,6-tetrachlorophenol.

Representative chromatograms for these studies are illustrated in Figures 1 and 2. Tables 5, 6 and 7 list actual analytical results from the PCP and HCB feeding studies. Note the difference in rat metabolism of PCP from that of HCB. Pentachlorothiophenol and 2,3,5,6-tetrachlorophenol appear to be metabolites of HCB but not PCP.

The developed methodology was then applied to human urine samples. Table 8 is a listing of these results. As can be seen from the table, the levels of the two dihydroxy compounds in the urine of the occupationally

Table 2. Recoveries of Metabolites from Fortified Urine[1]

Metabolite	ppm Added	% Range	Avg. % Rec.	% Rel. Std. Dev.
2,3,5,6-tetra-chlorophenol	1.0	89.3-92.3	91.1	±1.3
	0.3	85.7-92.3	88.8	±2.7
	0.1	82.0-87.9	85.3	±2.5
	0.03	78.0-85.6	82.3	±3.3
	0.01	79.1-87.5	82.8	±3.6
2,3,4,6-tetra chlorophenol	1.0	88.9-92.4	90.9	±1.5
	0.3	86.1-91.8	88.9	±2.4
	0.1	83.1-88.3	86.0	±2.5
	0.03	80.8-84.2	82.5	±1.7
	0.01	79.6-86.8	82.6	±3.2
2,3,4,5-tetra-chlorophenol	1.0	89.3-95.6	93.1	±2.7
	0.3	89.0-94.3	91.8	±2.3
	0.1	86.0-91.0	88.2	±2.1
	0.03	85.8-90.3	87.4	±2.0
	0.01	82.8-90.5	85.6	±3.4
Pentachloro-phenol	1.0	95.2-97.8	96.5	±1.1
	0.3	91.5-95.2	93.4	±1.8
	0.1	86.0-95.0	92.0	±4.1
	0.03	93.8-100.4	97.2	±2.8
	0.01	90.6-96.3	93.2	±2.5
Tetrachloro-pyrocatechol	1.0	78.6-81.4	80.1	±1.2
	0.3	78.7-85.7	81.6	±2.9
	0.1	76.3-83.0	79.8	±3.2
	0.03	59.1-71.4	65.6	±5.4
	0.01	60.1-69.7	63.7	±4.3
Tetrachloro-hydroquinone	1.0	80.2-82.7	81.5	±1.0
	0.3	77.0-84.5	81.4	±3.2
	0.1	77.0-84.0	80.9	±2.9
	0.03	75.6-86.0	80.4	±4.6
	0.01	71.3-77.3	74.6	±2.5
Pentachloro-thiophenol	1.0	69.9-73.6	71.9	±1.5
	0.3	69.3-73.3	71.1	±1.7
	0.1	61.0-73.0	66.4	±6.0
	0.03	49.8-57.2	53.3	±3.3
	0.01	41.5-51.3	47.3	±4.2

[1]Four determinations for each.

Table 3. Recoveries of Methylated Metabolites of HCB and PCP from an Acid Alumina Column

Metabolite	Amount added, µg	Amount recovered, µg	% Recovery
2,3,4,6-tetrachlorophenol[a]	5.00	4.650	93.0
	1.00	0.970	97.0
	0.10	0.092	92.0
2,3,5,6-tetrachlorophenol[a]	5.00	4.723	94.5
	1.00	0.970	97.0
	0.10	0.093	93.0
Pentachlorophenol[a]	5.00	4.800	96.0
	1.00	0.930	93.0
	0.10	0.094	94.0
Pentachlorothiophenol[a]	5.00	4.850	97.0
	1.00	0.880	88.0
	0.10	0.091	91.0
2,3,4,5-tetrachlorophenol[b]	5.00	4.720	94.4
	1.00	0.893	89.3
	0.10	0.091	91.0
Tetrachloropyrocatechol[b]	5.00	4.700	94.0
	1.00	0.950	95.0
	0.10	0.095	95.0
Tetrachlorohydroquinone[b]	5.00	4.850	97.0
	1.00	0.960	96.0
	0.10	0.091	91.0

[a]Fraction 1 -- 20 ml 10% benzene in hexane

[b]Fraction 2 -- 20 ml 40% benzene in hexane

Table 4. Relative Retention Data[a] for Methylated Metabolites of HCB and PCP

Metabolite	4% Se-30/6% OV-210	1.5% OV-17/1.95% QF1	5% OV-210	3% OV-1	3% Silar 10-C	5% DEGS[b]
2,3,4,6-tetrachlorophenol	0.23	0.33	0.24	0.22	0.36	1.21
2,3,5,6-tetrachlorophenol	0.23	0.33	0.24	0.22	0.37	1.13
2,3,4,5-tetrachlorophenol	0.38	0.51	0.46	0.34	0.46	1.66
Pentachlorophenol	0.46	0.55	0.49	0.44	0.68	2.58
Tetrachloropyrocatechol	0.45	0.55	0.52	0.42	0.88	--
Tetrachlorohydroquinone	0.48	0.56	0.59	0.42	0.83	--
Pentachlorothiophenol	0.95	1.06	1.00	0.91	1.59	--

[a]Retention time relative to Aldrin

[b]Underivitized phenols

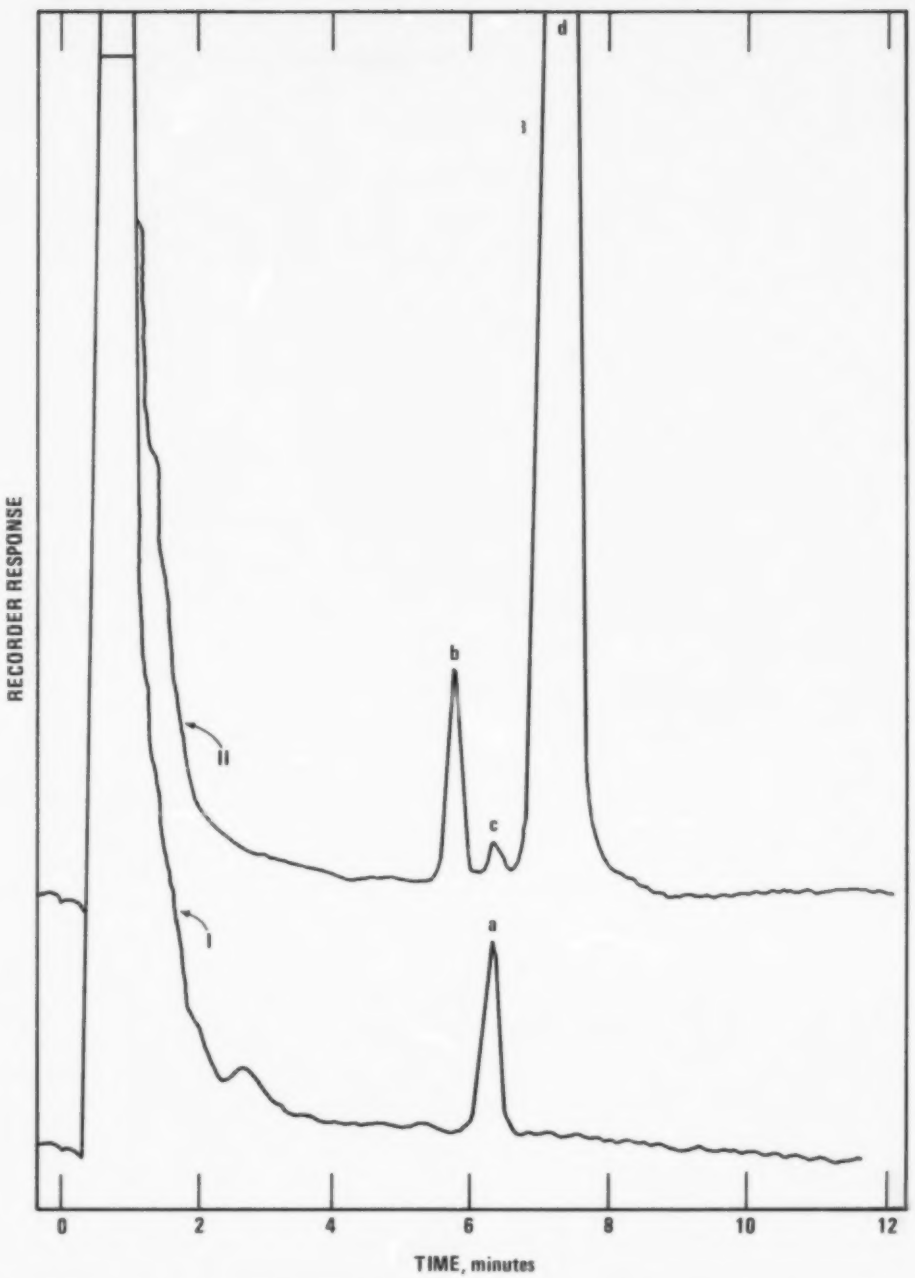

Figure 1. Gas chromatograms of urine extract from a rat fed PCP. (I) Fraction 1 acid alumina column. (a) PCP, 12.3 ppm; (II) Fraction II acid alumnia column. (b) 2, 3, 4, 5-tetrachlorophenol, 1.02 ppm; (c) tetrachlorophyrocatechol, 0.18 ppm; (d) tetrachlorohydroquinone, 24 ppm. Column: 5% OV-210 on 80/100 mesh Gas Chrom Q. Oven temperature 160°C. 5% methane in argon; flow rate 40 mL/min.

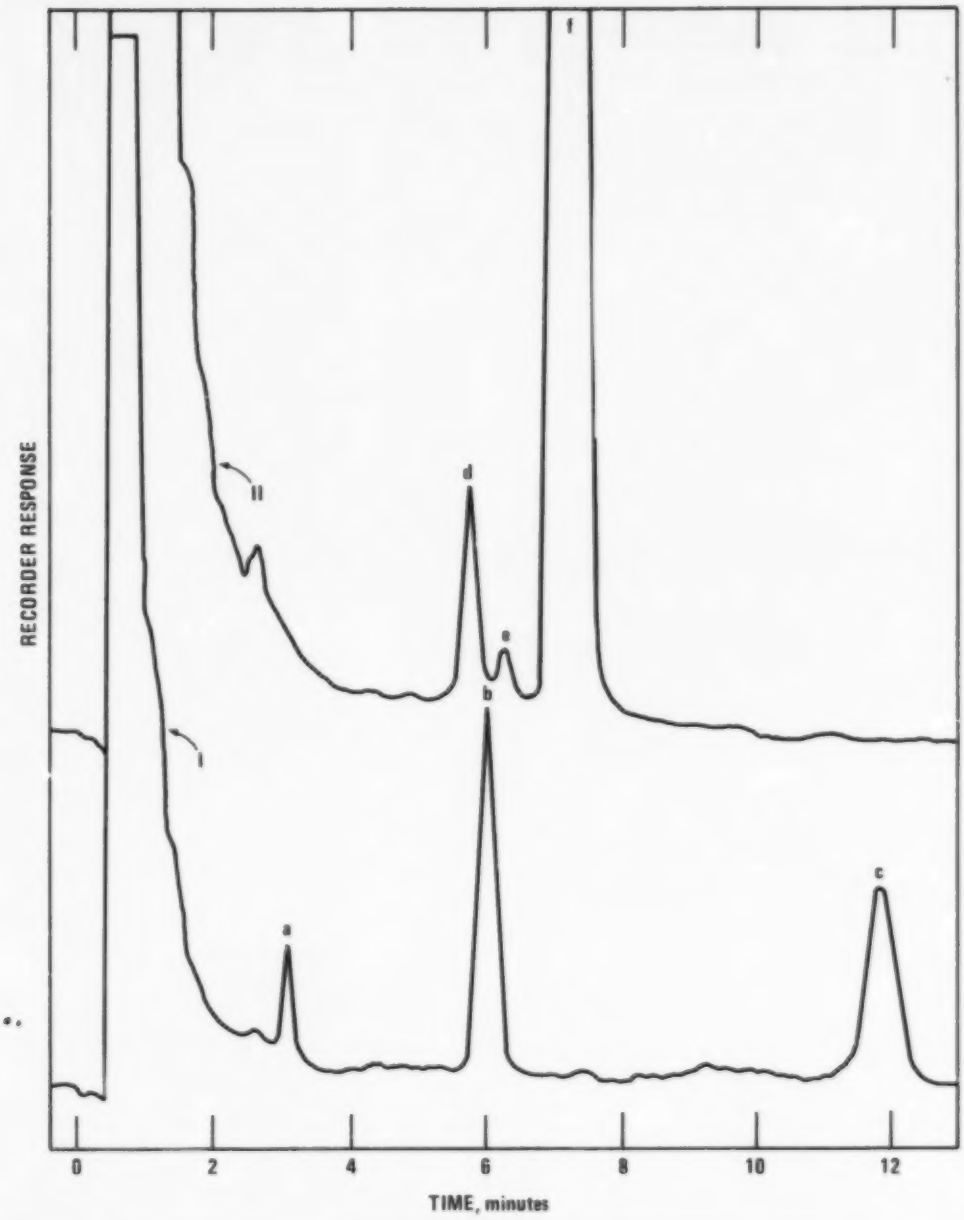

Figure 2. Gas chromatograms of urine extract from a rat fed HCB. (I) Fraction I acid alumina column. (a) 2, 3, 5, 6-tetrachlorophenol, 74 ppb; (b) PCP, 405 ppb; (c) pentachlorothiophenol, 500 ppb. (II) Fraction II acid alumina column; (d) 2, 3, 4, 5-tetrachlorophenol, 20 ppb; (e) tetrachloropyrocatechol, 8 ppb; (f) tetrachlorohydroquinone, 435 ppb. Column: 5% OV-210 on 80/100 mesh Gas Chrom Q. Oven temperature 160°C. 5% methane in argon; flow rate 40 mL/min.

Table 5. Hexachlorobenzene Metabolites in Rat Urine--100 ppm in Diet
Results in ppm

Days on Chow	Sample No.	Pentachloro-phenol	Tetrachloro-hydroquinone	Pentachloro-thiophenol	Tetrachloro-pyrocatechol	2,3,5,6-tetra-chlorophenol	2,3,4,5-tetra-chlorophenol
30	2240	0.081	0.079	0.424	0.001	0.029	0.005
107	2240	0.405	0.182	0.500	0.003	0.074	0.008
30	2241	0.167	0.238	0.390	0.002	0.033	0.013
107	2241	0.420	0.413	0.303	0.007	0.074	0.015
30	2242	0.085	0.031	0.560	0.001	0.027	0.001
107	2242	0.355	0.339	0.136	0.004	0.048	0.010
30	2243	0.066	0.027	0.485	0.001	0.025	0.002
107	2243	0.189	0.090	0.200	0.002	0.055	0.004
30	2244	0.079	0.114	0.332	0.002	0.023	0.005
107	2244	0.412	0.170	0.197	0.002	0.044	0.008
30	2245	0.168	0.139	0.747	0.002	0.042	0.005
107	2245	0.471	0.349	0.363	0.007	0.085	0.012

Table 6. Pentachlorophenol Metabolites in Rat Urine--100 ppm in Diet
Results in ppm

Days on Chow	Sample No.	Pentachloro-phenol	Tetrachloro-hydroquinone	Pentachloro-thiophenol	Tetrachloro-pyrocatechol	2,3,5,6-tetra-chlorophenol	2,3,4,5-tetra-chlorophenol
30	2246	16.8	48.8	<0.001	0.16	<0.001	2.17
107	2246	12.3	24.0	<0.001	0.18	<0.001	1.02
30	2247	9.2	25.9	<0.001	0.15	<0.001	1.05
107	2247	10.8	28.4	<0.001	0.17	<0.001	0.93
30	2248	12.2	40.4	<0.001	0.15	<0.001	1.33
107	2248	6.6	10.8	<0.001	0.08	<0.001	0.36
30	2249	21.0	42.8	<0.001	0.11	<0.001	1.18
107	2249	10.1	20.4	<0.001	0.16	<0.001	1.00
30	2250	19.4	38.4	<0.001	0.40	<0.001	1.48
107	2250	15.3	29.7	<0.001	0.42	<0.001	1.31
30	2251	7.4	27.0	<0.001	0.31	<0.001	1.19
107	2251	8.1	14.3	<0.001	0.28	<0.001	0.87

Table 7. Control Urine--Plain Chow
Results in ppm

Days on Chow	Sample No.	Pentachloro-phenol	Tetrachloro-hydroquinone	Pentachloro-thiophenol	Tetrachloro-pyrocatechol	2,3,5,6-tetra-chlorophenol	2,3,4,5-tetra-chlorophenol
30	2236	0.002	0.008	<0.001	0.004	<0.001	<0.001
107	2236	0.004	0.006	<0.001	0.002	<0.001	<0.001
30	2237	0.003	0.004	<0.001	0.002	<0.001	0.001
107	2237	0.002	0.012	<0.001	0.002	<0.001	0.004
30	2238	0.033	0.006	<0.001	<0.001	<0.001	0.015
107	2238	0.021	0.011	<0.001	0.003	<0.001	0.021
30	2239	0.041	0.037	<0.001	0.007	<0.001	0.011
107	2239	0.036	0.043	<0.001	0.009	<0.001	0.008

exposed worker are in nearly equal proportion. This may indicate a possible path for human metabolism of PCP and also be used as a further indicator of exposure to this compound.

Pentachlorophenol was identified in ten of the eleven urine samples from the general human population using the described analytical methodology, and ranged from 1 to 80 ppb. The presence of 2,3,4,6-tetrachlorophenol in the urine can possibly be attributed to its presence as an impurity in preparations of PCP. The only measurable metabolites from the general human population samples were tetrachlorohydroquinone and tetrachloropyrocatechol. A representative chromatogram from the urine of an occupationally exposed worker is shown in Figure 4. The occupationally exposed worker contained a high level of PCP and measurable levels of tetrachlorohydroquinone and tetrachloropyrocatechol.

As can be seen from these results, pentachlorothiophenol in urine can be used as an indicator of possible exposure to HCB. PCP exposure would be indicated by a high level of PCP and the presence of tetrachlorohydroquinone and tetrachloropyrocatechol in the urine.

CONFIRMATION OF METABOLITES BY GC/MS

The phenolic metabolites in urine extracts from the feeding study and human population were confirmed as their methyl ethers by combined GC-MS. Chemical ionization, using methane reagent gas, produced fairly strong M + 1 protonated-molecular ion cluster beginning at m/z 245 for the three isomers of tetrachlorophenol, m/z 279 for pentachlorophenol,

m/z 295 for pentachlorothiophenol and m/z 275 for tetrachloropyrocatechol and tetrachlorohydroquinone. In addition, a fairly strong M + 1 protonated molecular ion isotope cluster beginning at m/z 241 was tentatively identified as an isomer of the methyl ether of trichlorodihydroxybenzene from the PCP feeding study samples. The phenolic metabolites in the urine from the occupationally exposed worker were confirmed by GC/MS as the methyl ethers of tetrachloropyrocatechol and tetrachlorohydroquinone. The structures of the metabolites isolated and confirmed from the HCB/PCP feeding studies are shown in Figures 4 and 5.

Table 8. General Human Population Urine Samples - Results in ppm

Sample No.	Pentachlorophenol	Tetrachlorohydroquinone	Pentachlorothiophenol	Tetrachloropyrocatechol	2,3,4,6-tetrachlorophenol	2,3,5,6-tetrachlorophenol	2,3,4,5-tetrachlorophenol
1	0.006	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
2	0.012	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
3	0.004	<0.001	<0.001	0.002	0.003	<0.001	<0.001
4	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	0.080	0.002	<0.001	0.001	0.013	<0.001	<0.001
6	0.004	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
7	0.015	0.003	<0.001	0.004	0.004	<0.001	<0.001
8	0.012	0.006	<0.001	0.005	0.002	<0.001	<0.001
9	0.009	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
10	0.038	0.008	<0.001	0.007	0.009	<0.001	<0.001
11	0.018	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
12*	3.60	0.024	<0.001	0.024	0.123	<0.001	0.005

*Occupationally exposed to PCP

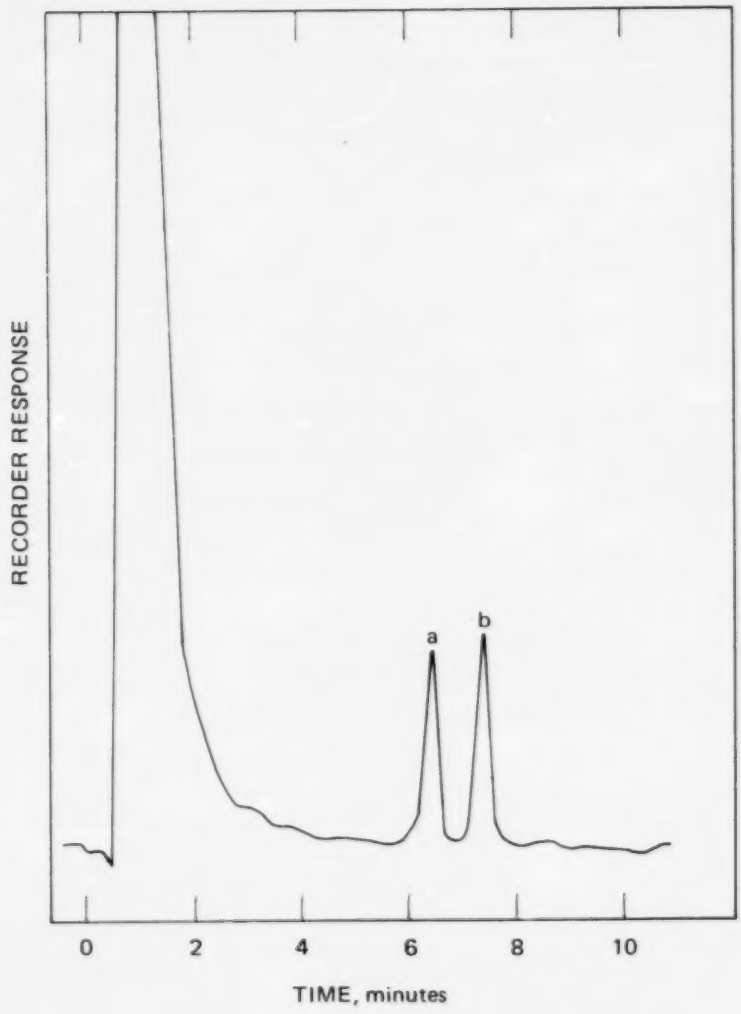

Figure 3. Gas chromatogram of Fraction 2, Acid Alumina Column. 2 ml urine extract, PCP exposed worker (a) tetrachloropyrocatechol, equiv. 24 ppb; (b) tetrachlorohydroquinone, equiv. 24 ppb. 5% OV-210, 160°C, 40 mL/min.

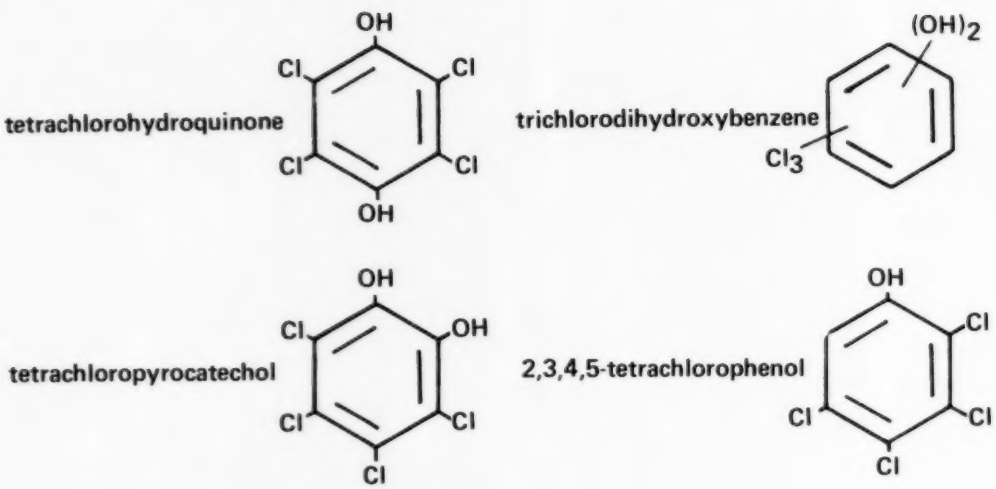

Figure 4. Metabolites isolated and confirmed from PCP feeding study.

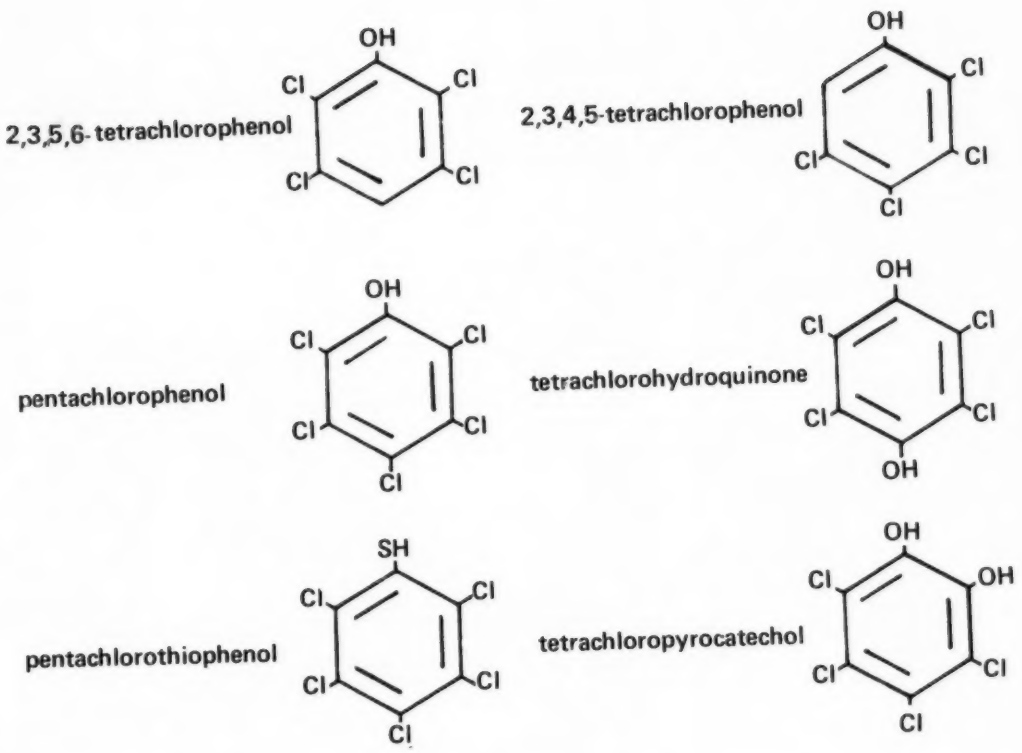

Figure 5. Metabolites isolated and confirmed from HCB feeding study.

ANALYSIS FOR UNDERIVATIZED CHLORINATED PHENOLS

Relative retention times of the chlorinated phenols analyzed as the free phenol are presented in Tables 9 and 10. The difference in elution patterns of SB-BDS from that of DSB-DEGS allows confirmation of chlorinated phenols in urine. A double support-bonded DEGS column packing was chosen over conventional and single support-bonded packings because of its efficiency, low bleed and longevity. The column was prepared by double heat treating stabilized diethylene glycol succinate on acid washed Chromosorb W.[18,19] A 0.6 m column was used for the rapid elution of pentachorophenol. Work is continuing in our laboratory on the use of HPLC for the separation and confirmation of chlorinated phenols. These results will be presented in a later publication.

As shown in Table 11, recoveries of chlorinated phenols from fortified urine averaged better than 80%. Method sensitivity is dependent upon chlorine substitution and elution time of the phenol through the gas chromatographic column. Method sensitivity for di- and trichlorophenols (except 3,4- and 3,5-dichlorophenol and 3,4,5-trichlorophenol) is 1 ppb. The method sensitivity for the tetrachlorophenols and pentachlorophenol is 2 ppb. Because of the possible effect of meta-meta substitution, method sensitivity for 3,5-dichlorophenol is 15 ppb and 150 ppb for 3,4-dichlorophenol and 3,4,5-trichlorophenol.

Table 12 lists results from the application of this analytical methodology to general population human urine samples. (Different samples from those of Table 8.) Only those chlorinated phenols that had identical retention times with standards on DSB-DEGS and SB-BDS are

Table 9. Retention Data for Chlorinated Phenols on a Double Support-Bonded DEGS Column*

Compound	minutes	$RRT_{2,4,5\text{-TCP}}$
2,6-dichlorophenol	2.95	0.35
2,4-dichlorophenol	3.19	0.37
2,5-dichlorophenol	3.19	0.37
2,3-dichlorophenol	3.39	0.40
2,4,6-trichlorophenol	5.47	0.64
2,3,5-trichlorophenol	6.81	0.80
2,3,6-trichlorophenol	6.85	0.80
2,4,5-trichlorophenol	8.54	1.00
2,3,4-trichlorophenol	8.94	1.05
2,3,5,6-tetrachlorophenol	13.15	1.54
2,3,4,6-tetrachlorophenol	14.84	1.74
3,5-dichlorophenol	19.29	2.26
3,4,5-trichlorophenol	20.47	2.40
2,3,4,5-tetrachlorophenol	21.18	2.48
3,4-dichlorophenol	25.83	3.02
pentachlorophenol	35.20	4.12

*Double Support-Bonded DEGS - 1.8 m x 4 mm; 170°C, 60 ml/min.

Table 10. Retention Data for Chlorinated Phenols on a Support-Bonded BDS Column*

Compound	minutes	$RRT_{2,4,5\text{-TCP}}$
2,6-dichlorophenol	0.98	0.30
2,4-dichlorophenol	0.98	0.30
2,5-dichlorophenol	1.02	0.31
2,3-dichlorophenol	1.02	0.31
2,4,6-trichlorophenol	2.83	0.86
2,3,5-trichlorophenol	2.83	0.86
2,3,4-trichlorophenol	3.15	0.95
2,4,5-trichlorophenol	3.31	1.00
2,3,6-trichlorophenol	4.25	1.29
3,5-dichlorophenol	6.69	2.02
3,4-dichlorophenol	8.19	2.48
2,3,4,5-tetrachlorophenol	11.10	3.36
2,3,4,6-tetrachlorophenol	17.56	5.31
3,4,5-trichlorophenol	24.62	7.26
2,3,5,6-tetrachlorophenol	27.76	8.39
pentachlorophenol	--	--

*Support-Bonded BDS - 0.6 m x 4 mm; 190°C, 60 mL/um

Table 11. Recoveries of Chlorinated Phenols from Fortified Urine

Compound	ppm added* 0.01 Av % Rec	0.01 % SD	0.05 Av % Rec	0.05 % SD	0.10 Av % Rec	0.10 % SD	0.50 Av % Rec	0.50 % SD	1.00 Av % Rec	1.00 % SD	Range**
2,6-dichlorophenol	83	± 7.7	96	± 5.6	90	± 9.0	83	± 2.5	84	± 5.3	75-104
2,4-dichlorophenol	86	± 12.3	89	± 5.3	83	± 7.4	84	± 2.8	86	± 4.1	70-103
2,3-dichlorophenol	86	± 3.4	83	± 4.2	87	± 3.2	89	± 3.8	88	± 3.8	78-92
2,5-dichlorophenol	80	± 1.3	83	± 3.0	89	± 4.0	84	± 2.4	86	± 4.1	79-92
3,4-dichlorophenol			86	± 6.3	87	± 4.0	87	± 4.5	86	± 4.9	80-96
3,5-dichlorophenol			82	± 3.2	81	± 1.9	81	± 2.3	82	± 1.6	77-88
2,3,4-trichlorophenol	85	± 4.6	87	± 6.3	88	± 4.4	89	± 7.6	88	± 4.0	80-101
2,3,5-trichlorophenol	80	± 2.8	81	± 5.4	85	± 4.2	87	± 7.5	86	± 3.8	74-100
2,3,6-trichlorophenol	88	± 7.7	92	± 7.5	90	± 7.3	90	± 5.6	83	± 6.7	79-102
2,4,5-trichlorophenol	87	± 5.0	89	± 6.6	81	± 3.8	86	± 3.1	86	± 4.9	76-94
2,4,6-trichlorophenol	85	± 15.0	98	± 8.9	93	± 5.7	84	± 2.9	88	± 7.5	70-110
3,4,5-trichlorophenol			82	± 4.3	86	± 4.6	84	± 2.8	86	± 5.6	78-92
2,3,5,6-tetrachlorophenol	87	± 5.3	91	± 9.2	88	± 7.2	87	± 6.7	92	± 7.6	75-106
2,3,4,6-tetrachlorophenol	87	± 3.6	94	± 7.7	92	± 5.3	88	± 5.1	94	± 6.0	81-102
2,3,4,5-tetrachlorophenol	84	± 6.8	92	± 10.3	82	± 7.1	91	± 6.7	97	± 4.7	74-102
pentachlorophenol	79	± 5.6	86	± 4.9	85	± 4.9	88	± 5.2	93	± 5.1	70-101

*Av. 5 determinations each fortification level.
**Range for all fortification levels.

listed. In addition, the presence and levels of chlorinated phenols in pooled urine samples were confirmed by HPLC/MS. These samples were taken from individuals who have no known exposure which can give chlorinated phenols as metabolites in urine. These results allow for a background or control level of expected chlorophenols in urine.

Figure 6 and 7 are representative chromatograms for a general human urine sample free phenol analysis. As can be seen from the chromatographs, a wide variety of GC operating parameters can be employed to analyze for both early and late eluting chlorophenols.

Table 12. General Human Population Urine Sample - Chlorinated Phenols Residues in ppb

Sample Number	2,6-dcp	2,5-dcp	3,5-dcp	2,4,6-tcp	2,4,5-tcp	2,3,4,6-tcp	PCP
1	<1	21	<15	2	7	2	7
2	<1	13	<15	6	7	4	9
3	38	20	<15	<1	7	2	4
4	<1	11	<15	1	2	2	6
5	<1	14	<15	1	8	3	11
6	<1	21	<15	1	3	2	14
7	<1	6	<15	1	3	2	8
8	<1	161	<15	4	9	15	74
9	<1	100	16	3	6	3	23
10	<1	10	<15	<1	<1	3	9
11	31	2	<15	2	2	<2	5
12	112	30	44	3	5	3	8
13	14	81	20	1	8	4	15
14	31	117	<15	3	3	6	11
15	76	159	29	<1	4	27	29
16	11	71	53	<1	<1	7	19
17	31	6	16	<1	17	5	11
18	<1	45	42	<1	2	8	16
19	21	117	21	<1	4	6	11
20	31	15	<15	<1	3	6	9
21	<1	13	<15	<1	2	4	3
22	40	132	<15	<1	5	7	6
23	<1	13	<15	<1	<1	3	5
24	<1	156	18	<1	37	9	9
25	<1	9	36	<1	4	7	14
26	<1	179	<15	<1	<1	6	13
27	<1	25	30	<1	<1	3	6
28	<1	23	11	2	4	7	8
29	<1	33	19	4	8	14	5
30	<1	56	16	5	16	4	26
31	<1	30	11	3	5	15	18
32	18	104	6	2	2	5	25
33	<1	208	10	4	18	6	25
34	12	35	9	5	5	9	28

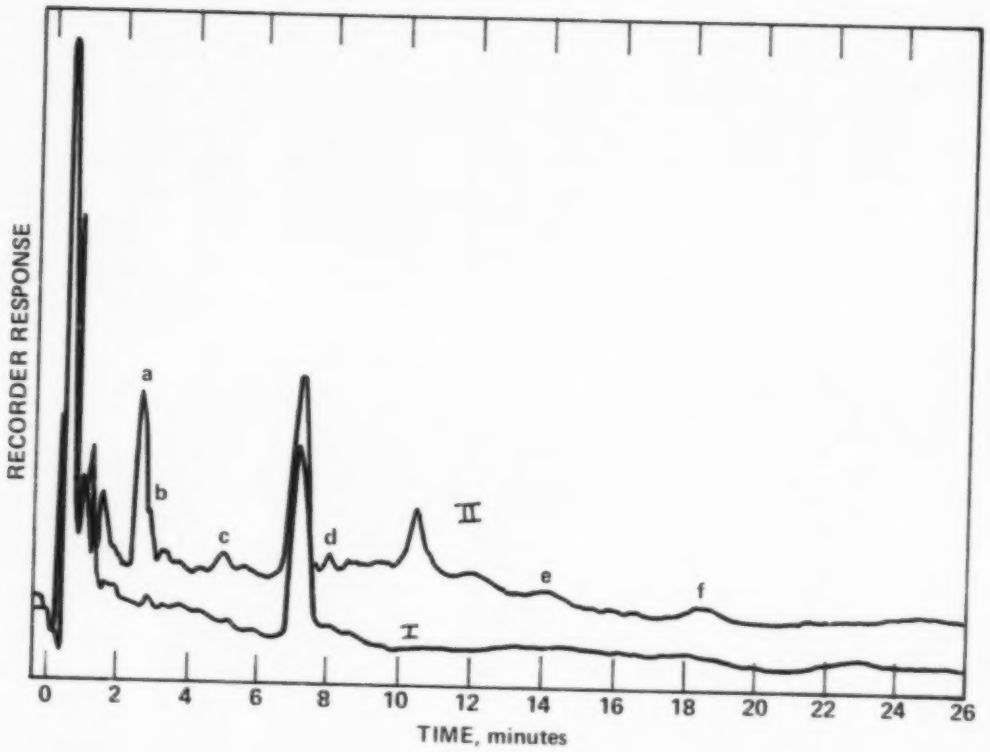

Figure 6. Chromatograms of (I) XAD-4 reagent blank and (II) human urine: (a) 2, 6-dichlorophenol, equiv. 112 ppb; (b) 2, 4 - and/or 2, 5-dichlorophenol, equiv. 30 ppb; (c) 2, 4, 6-trichlorophenol, equiv. 3 ppb; (d) 2, 4, 5-trichlorophenol, equiv. 5 ppb; (e) 2, 3, 4, 6-tetrachlorophenol, equiv. 3 ppb; (f) 3, 5-dichlorophenol, equiv. 44 ppb. Column: DSB - DEGS (1.8 x 4 mm 1.0) on 80/100 chrom W (AW); oven temperature, 170°C; 5% methane in argon; flow rate 60 mL/min.

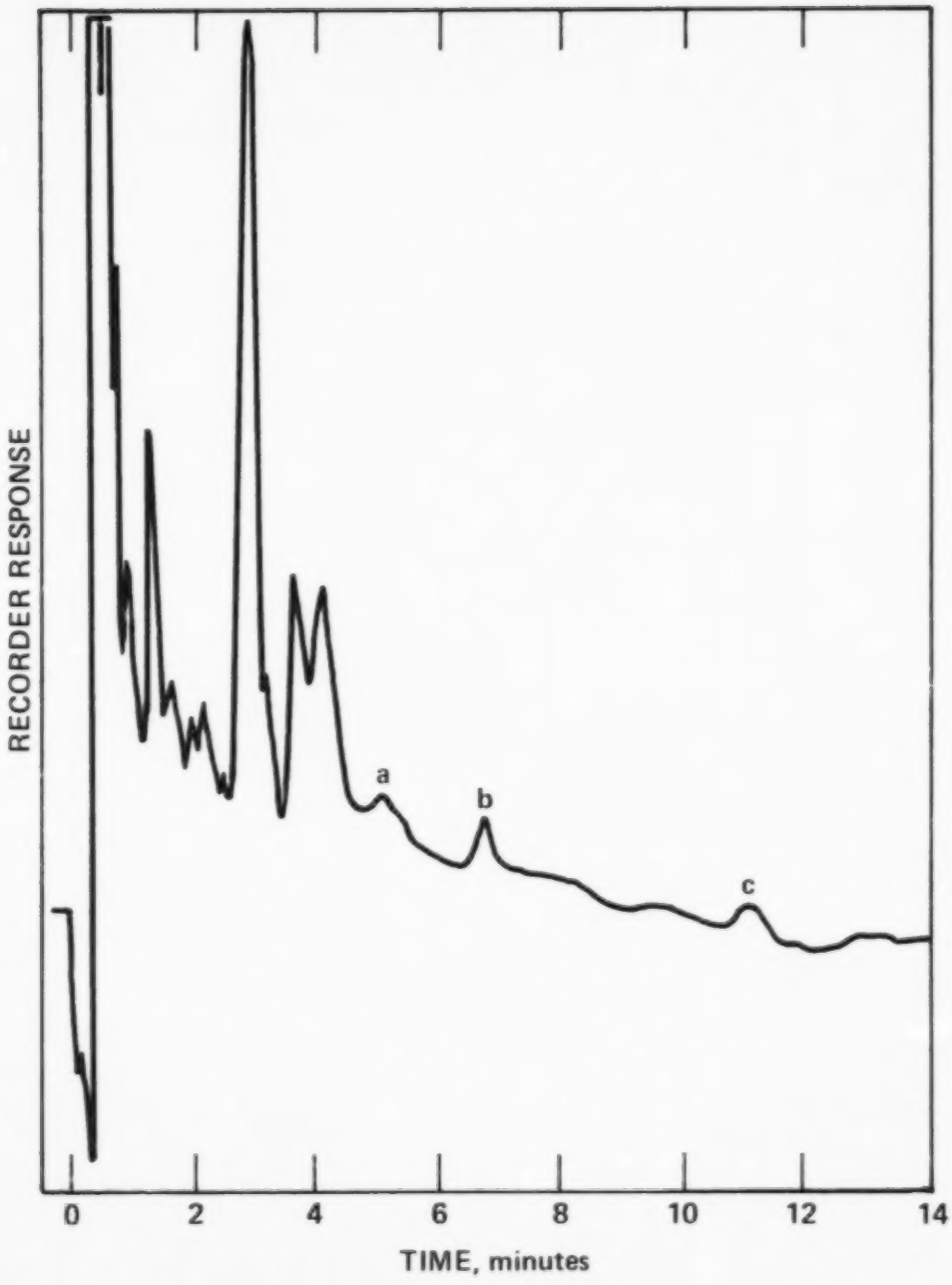

Figure 7. Chromatogram of human urine. (e) 2, 3, 4, 5-tetrachlorophenol, equiv. 3 ppb; (f) 3, 5-dichlorophenol, equiv. 40 ppb; (g) pentachlorophenol, equiv. 8 ppb. Column: DSB - DEGS (0.6 m x 4 mm I. D.) on 80/100 Chrom W (AW); oven temperature 195°C; 5% methane in argon; flow rate 60 mL/min.

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